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Volkensin, the toxin of *Adenia volkensii* (kilyambiti plant)

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A toxic protein, volkensin, has been purified from the roots of *Adenia volkensii*. This toxin is a galactose-specific lectin with M_r 62000, consisting of two subunits with M_r 36000 and 29000. Volkensin inhibits protein synthesis by a lysate of rabbit reticulocytes and by HeLa cells, and is highly toxic to mice (LD_{50} 1.38 μg/kg body wt).

Adenia volkensii *Volkensin* *Toxin* *Lectin* *Protein-synthesis inhibition*

1. INTRODUCTION

Adenia volkensii Harms (kilyambiti plant), a member of the Passifloraceae, is a perennial shrub growing wild in several areas of Kenya. The plant is known to be highly poisonous, and is used locally to kill hyaenas [1]. Its toxicity was attributed to the HCN liberated from a cyanogenetic glycoside contained in the plant [2], an hypothesis, however, not consistent with the delayed deaths and with the lesions observed in poisoned animals. The author in [3] partially purified toxic proteins from the roots of *A. volkensii* and of *Adenia digitata* (*Modecca digitata*), a related plant from which a potent toxin, modeccin, was isolated [4,5].

We here purified from the roots of *A. volkensii* a galactose-specific lectin which is a very potent toxin similar to modeccin, and for which the name of volkensin is proposed.

2. EXPERIMENTAL

2.1. *Materials*

Roots of *A. volkensii* were obtained from the University Herbarium, Nairobi, Kenya. All chemicals were obtained from the same sources as in [6].

2.2. *Biochemical determinations*

M_r values were determined by SDS-polyacrylamide gel electrophoresis (SDS–PAGE) [7] or by gel filtration on Bio-Gel P-150. Protein was determined as in [8] with bovine serum albumin as a standard.

Haemagglutinating activity was determined as in [9] with trypsinized [10] rabbit erythrocytes fixed with glutaraldehyde [11].

Protein synthesis was determined with a lysate of rabbit reticulocytes and with HeLa cells as in [6,12].

2.3. *Toxicity experiments*

The toxicity of volkensin was evaluated in male Swiss mice weighing 20 g, receiving food and water ad libitum. The toxin, dissolved in 0.9% NaCl, was injected intraperitoneally at 5 doses varying from 0.1 to 10 μg/100 g body wt, to groups of 5 animals/dose. LD_{50} at 14 days was calculated by the method of Spearman-Kärber as in [13].

3. RESULTS

3.1. *Purification of volkensin*

Roots of *A. volkensii* were peeled, minced, and homogenized with 5 ml/g of 0.14 M NaCl containing 0.005 M Na–phosphate buffer (pH 7.2) and 0.1 M cystamine (to prevent release of HCN). The

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homogenate was stirred overnight at 2–4°C. Fibres were removed by centrifugation in a kitchen centrifuge for vegetables, and the cloudy liquid obtained was centrifuged at 20000 × *g* for 30 min. To the clear supernatant solid ammonium sulphate to 100% saturation was added slowly under constant stirring. The precipitate was collected by centrifugation, redissolved in phosphate-buffered same solution for at least 24 h. A precipitate formed during dialysis was removed by centrifugation, and the clear extract was applied to a column of acid-treated [14] Sepharose 6B, previously acid-treated [14] Sepharose 6B, previously equilibrated with phosphate-buffered saline, at 4°C. A single, sharp peak of protein, henceforth referred to as volkensin, was eluted with 0.2 M galactose in phosphate-buffered saline. Volkensin was dialysed extensively against water, freeze-dried, and stored at −25°C. The yield was 40–75 mg/100 g roots.

3.2. *Properties of volkensin*

On polyacrylamide gel electrophoresis, volkensin showed a major band with M_r 60000, and two smaller bands with M_r 36000 and 29000. After reduction of the toxin with 1% 2-mercaptoethanol for 1 h at 37°C, the heavier band disappeared and only the two lighter bands were visible. The toxin gave a single peak on gel filtration both on Bio-Gel P-150 (M_r 62000) and on Sephacryl S-200 superfine.

Volkensin agglutinated trypsinized rabbit erythrocytes at the minimum concentration of 1 μg/ml. Agglutination was prevented in the presence of 6 mM D-galactose.

The toxin inhibited protein synthesis by a lysate of rabbit reticulocytes and by HeLa cells. This effect was abolished if the toxin was heated at 75°C for 25 min. If the toxin was reduced with 2-mercaptoethanol the inhibitory effect on the lysate system was strongly enhanced, whereas that on cells was abolished (table 1). The inhibitory effect on the lysate was unchanged in the presence of 25 mM D-galactose, whereas the inhibition of protein synthesis by HeLa cells was abolished by 10 mM lactose (not shown).

Volkensin was highly toxic to mice, with an LD_{50} of 1.38 μg/kg body wt (95% confidence limits 0.56–3.36 μg/kg). Deaths never occurred before 8 h of poisoning.

Table 1

Effect of volkensin on protein synthesis

Additions	ID_{50}[a] (μg/ml)	
	Reticulocyte lysate	HeLa cells
Volkensin	5.0	7.6×10^{-4}
Reduced volkensin	2×10^{-2}	No inhibition at 10^{-3}

[a] Concentration causing 50% inhibition

Protein synthesis in the lysate system was evaluated as described in the text. Reaction mixtures (62.5 μl) contained 10 mM Tris–HCl buffer (pH 7.4), 100 mM ammonium acetate, 2 mM magnesium acetate, 1 mM ATP, 0.2 mM GTP, 15 mM phosphocreatine, 3 μg creatine kinase, 0.05 mM amino acids (minus leucine), 0.095 μCi L-[^{14}C]leucine, 25 μl rabbit reticulocyte lysate. Incubation was at 28°C for 5 min. The reaction was stopped with 1 ml of 0.1 M KOH, and the radioactivity incorporated into protein was measured as in [6]. Protein synthesis by HeLa cells was evaluated with 10^5 cells grown in 1 ml of RPMI medium containing 10% human AB serum. Cells were incubated for 18 h at 37°C in a humidified atmosphere of air/CO_2 (19:1) in the presence of various concentrations of volkensin. The medium was then removed and replaced with 1 ml of RPMI medium containing 0.25 μCi L-[^{14}C]leucine. After incubation for 2 h the medium was removed and replaced with 1 ml of 0.1 M KOH. Protein was precipitated with 1 ml of 20% trichloroacetic acid and treated as in [12]

4. DISCUSSION

Our results demonstrate that the roots of *A. volkensii* contain a toxic protein, volkensin, the potency of which may well account for the poisonous effect of the plant.

The toxin appears to be a galactose-specific lectin, since: (i) it binds to Sepharose and can be eluted with galactose; and (ii) it agglutinates erythrocytes, and this effect is inhibited by galactose.

Volkensin inhibits protein synthesis by a reticulocyte lysate as well as by intact cells. The inhibitory effect on the cell-free system is independent of the lectin properties of volkensin, whereas lactose abolishes the effect on protein synthesis by cells. This suggests that entry of the toxin into cells

occurs through binding to galactosyl-terminated receptors on the cell membrane.

For all its properties volkensin seems very similar to ricin and related toxins (abrin, modeccin and viscumin; review [15]), which all bind galactose and inhibit protein synthesis. Like these toxins, volkensin consists of two unequal subunits, joined by one (or more) disulphide bond(s), the reduction of which enhances the effect of the toxin on cell-free protein synthesis, whilst decreasing that on intact cells, as was also observed with ricin and similar toxins [4,16–18]. Thus it seems likely that the subunits of volkensin correspond to the A and B subunits of ricin, which both concur to, and are required for, the toxic effect of this substance, the former by damaging ribosomes, thus inhibiting protein synthesis, and the latter by binding to receptors on the cell membrane, thus allowing entry into the cytoplasm.

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